Reference: *Biol. Bull.*, 159: 231–246. (August, 1980)

THE ZOOGEOGRAPHY AND DIETARY INDUCTION OF BIOLUMINESCENCE IN THE MIDSHIPMAN FISH, *PORICHTHYS NOTATUS*

JON A. WARNER[1] AND JAMES F. CASE

Department of Biological Sciences, University of California, Santa Barbara, California 93106

The light generating systems of *Porichthys notatus*, the midshipman fish, cross react with those of the ostracod *Vargula* (= *Cypridina*) *hilgendorfii* (Cormier *et al.*, 1967), indicating that *Porichthys* might be able to utilize exogenous luciferin to support luminescence. This remarkable possibility is not wholly unexpected since some fishes in the two genera *Apogon* and *Parapriacanthus* are thought to acquire their luciferin from a sympatric ostracod, *V. hilgendorfii* (Haneda *et al.*, 1966, 1969; Tsuji *et al.*, 1971). The research reported here reinforces possible dependence of the midshipman on an exogenous luciferin.

P. notatus occurs in coastal waters from Baja California to Southeastern Alaskan waters (Wilimovski, 1954), a range encompassing many kinds of luminescent organisms which might furnish luciferin (Tsuji *et al.*, 1971). However, luminescent ostracods, the most likely dietary source of luciferin, were not known to occur within the range of the midshipman until Kornicker and Baker (1977) described *Vargula tsujii* (Mycopoda; Cyprindinae). The existence of a luminescent ostracod closely related to *V. hilgendorfii* but, unlike it, sympatric with the southern midshipman population, heightens interest in the possibility that *Porichthys* might normally utilize an exogenous source of luciferin.

Midshipman luminescence is generated by an adrenergically triggered, intracellular, luciferase-mediated oxidation of luciferin in photocytes in hundreds of ventral and lateral photophores (Greene, 1899; Greene and Greene, 1924; Nicol, 1957; Baguet and Case, 1971; Baguet, 1975; Anctil, 1977, 1979a). Case and Strause (1979) and Anctil (1979b) proposed that the characteristic cytoplasmic vesicles and microvillous borders of the photocytes are specializations for uptake and storage of luciferin from large body stores identified by Tsuji *et al.*, 1971.

Specimens of *P. notatus* from Puget Sound are not bioluminescent (Strum, 1968) even though their photophores are morphologically and ultrastructurally similar to those of the southern luminescent fish (Strum, 1969a and b; Anctil and Case, 1976), except for possible reduced amounts of flocculent ground substance in cytoplasmic vesicles of nonluminescent specimens from Puget Sound (unpublished observations). Midshipman fish collected off Southern California contain luciferin in all body tissues, with larger concentrations in photophores (Tsuji *et al.*, 1971), while fish from southern Puget Sound are luciferin deficient, both as embryos and adults (Barnes *et al.*, 1973). Induction of luminescence capability in non-luminescent fish has been achieved with intraperitoneal injection of *V. hilgendorfii* luciferin (Tsuji *et al.*, 1972) and by force feeding of whole dried *V. hilgendorfii* (Barnes *et al.*, 1973). Either technique makes luminescence possible after about 4 days. The bioluminescent response to noradrenaline continues to increase for

[1] Present address: Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, California 92093.

TABLE I

Porichthys bioluminescence survey

Location	Date	Depth (m)	Collecting method	Total trawling time (hr)	Number fish	Size range (mm)	Sex ratio (♂/♀)	Fluorescence %/number	Bioluminescence %/number
Baynes Sound, B. C. 49° 30′ N/124° 48′ W	Aug. 77	0–30	intertidal	—	32	27–31 juveniles		0/32	0/32
					10	180–200 adults	1.00/0	0/10	0/10
Imperial Eagle Channel, B. C. 48° 51′ N/125° 15′ W	Aug. 77	100–120	50′ shrimp trawl	—	30	125–265	0.166/0.834	0/30	0/12
Saanich Inlet, B. C. 48° 34′ N/123° 30′ W	Oct. 76	200	—	—	7	181–243		0/7	0/7
Port Orchard, WA* 47° 45′ N/122° 30′ W	1971–1972		—	—	308			0/308	0/308
Yaquina Head, OR 44° 37′ N/124° 25′ W	Sep. 77	30–100	16′ otter trawl	6	0				
Yaquina Head, OR 44° 37′ N/124° 34′ W	Sep. 77	200	74′ shrimp trawl	24	0				
Yaquina Bay, OR 44° 36′ N/124° 00′ W	Sep. 77	2–10	16′ otter trawl	1	0				
Cape Arago, OR 43° 30′ N/124° 35′ W	Sep. 77	200	72′ shrimp trawl	6	0				
San Pablo St., SF Bay, CA 37° 57′ N/122° 26′ W	May 77	10–30	43′ otter trawl	4	65	75–310	0.75/0.25	85/65	85/65
Gulf of the Farallons, CA 37° 08′ N/124° 15′ W	Jun. 77	20–140	43′ otter trawl	4	281	85–300		85/281	
Moss Landing, CA 36° 51′ N/121° 55′ W	Jun. 77	12–100	16′ otter trawl	0.5	31	160–310		100/31	100/31
Pt. Cabrillo, Monterey, CA 36° 38′ N/121° 54′ W	Jun. 77	25	16′ otter trawl	0.25	39	81–198		100/39	100/15
Santa Barbara Channel, CA 34° 22′ N/119° 30′ W	Jun.–Nov. 77	3–20	scuba		83	27–330	0.99/0.01	100/83	100/41
Santa Monica Bay, CA 33° 55′ N/118° 42′ W	Mar. 77	500	15′ balloon trawl	—	12	160–210		100/12	100/1

* See Barnes *et al.*, 1973.

several weeks without further feeding but ultimately disappears after several months. Luminescence is inducible with very low doses of *V. hilgendorfii* luciferin, or feeding with only one whole, dried specimen. Depletion of luciferin stores does not occur after injections of norepinephrine, which produces long-lasting luminescence. Enzymatically- and air-oxidized *V. hilgendorfii* luciferin, synthetic oxyluciferin, and three luciferin analogs failed to induce luminescence in non-luminescent fish (Barnes *et al.,* 1973; Tsuji *et al.,* 1975).

There is a strong correlation between ultraviolet-stimulated photophore fluorescence and ability to luminesce in the midshipman. Photophores of midshipman fish from southern California waters emit green fluorescence when excited with 365 nm u.v. light. Specimens from Hood Canal (Puget Sound) neither luminesce nor fluoresce (Barnes *et al.,* 1973). Fluorescence is also undetectable in larval southern fish before the 28th day of development, while subsequently both fluorescence and luminescence are present (Anctil, 1977). Baguet and Ziets-Nicolas (1979) report that the intensity of photophore fluorescence decreases as a linear function of light emitted when isolated photophores are stimulated to luminesce with epinephrine, norepinephrine or KCN. Thus it appears that photophore fluorescence is a useful indicator of the presence of luciferin and of ability to luminesce.

In this paper we correlate the distribution of luminescent and non-luminescent forms of the midshipman with the distribution of *Vargula tsujii,* show that feeding with *Vargula* induces luminescence capability in non-luminescent *Porichthys,* and demonstrate that fish thus treated have behavioral control of their newly acquired luminescence.

Materials and Methods

Field studies

Specimens of *P. notatus* were taken from nine sites along the Pacific coasts of the United States and Canada (Table I, Fig. 1). Bottom trawls were made at 1–3 knots at depths from 5–500 m. Experimental fish were maintained in separate 40 l aquaria in the laboratory and supplied aerated, sand-filtered sea water at 14°–18°C.

Bioluminescence and fluorescence were measured on fish anesthetized in MS-222 (Sigma), 0.1 g/l sea water, applied until cessation of swimming (2–15 min). Photophore fluorescence was estimated using a 365-nm-emitting u.v. lamp (UVSL 25, Ultra Violet Products. Inc., San Gabriel, Ca.) Field recordings of luminescence were made from anesthetized fish after I.P. injections of 0.1 ml of 0.001 mM DL-arterenol HCl (Sigma) in fish saline (Young, 1933). During the 15 min after injection luminescence was measured continuously with a photomultiplier (EMI 9781B) positioned over the ventral thoracic photophores.

Laboratory studies

Luminescence was recorded in the laboratory by injecting fish as above or subcutaneously, and viewing either all ventral photophores or those at the subcutaneous injection site with a photomultiplier (EMI 9781B). Photomultiplier output was led to a Keithley 427 current amplifier and displayed on a polygraph (Grass, model 79D9).

Luminescence intensity was determined relative to an arbitrary standard with this apparatus, as shown in Figure 2. Bioluminescence was recorded for several

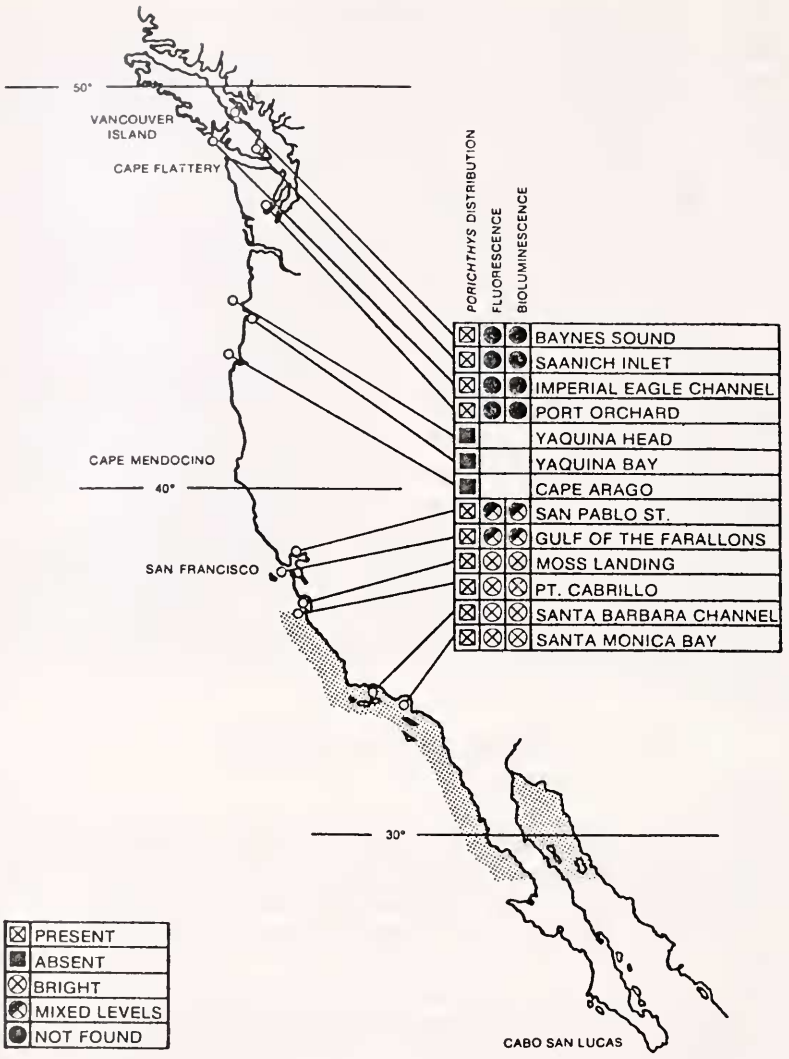

FIGURE 1. Collecting localities showing presence or absence of *Porichthys notatus* and the extent to which they exhibit luminescence and ultraviolet excited fluorescence. Shaded zone indicates distribution of *Vargula tsujii*.

minutes beginning immediately after a local subcutaneous injection of 0.4 ml of 0.001 nM DL-arterenol. A conical light guide, masked so as to limit recording to a single photophore of average size, served to control for the large variation in size of fish and photophores.

Feeding experiments

In attempts to induce luminescence by manipulating the diet, 15 (12–21 cm) non-luminescent fish from Saanich Inlet and Imperial Eagle Channel, Vancouver Island, Canada, were fed parts or whole individuals of several luminescent organisms sympatric with the southern, luminescent *P. notatus*. Organisms, details of preparation and effects on luminescence are shown in Tables II and III.

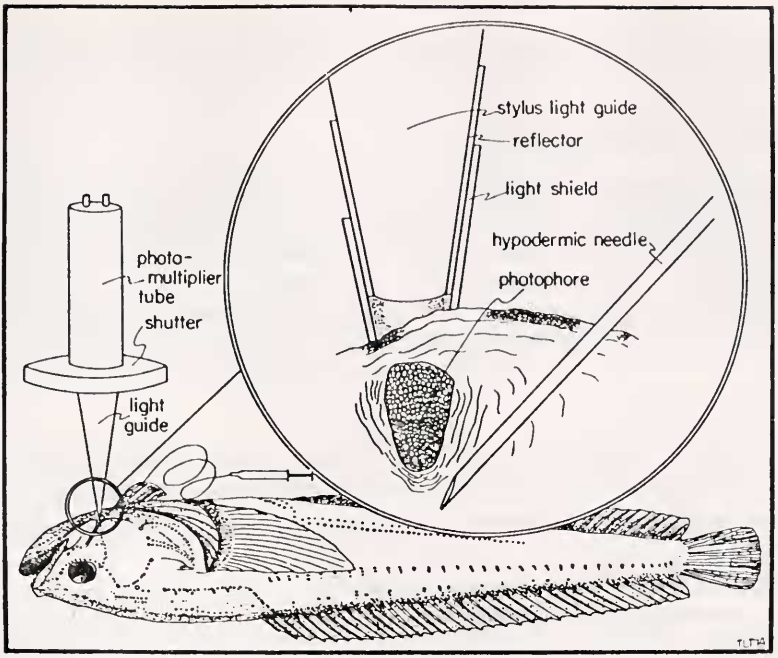

FIGURE 2. Arrangement used in recording luminescence from a single photophore in an intact *Porichthys*.

Anesthetized fish were fed by inserting an appropriate size gelatine capsule of food deep within the esophagus. Close observation during recovery from anesthesia guarded against regurgitation, a rare occurrence. Each feeding greatly exceeded

TABLE II

Materials used in luminescence induction experiments

Organism	Source	Method of preparation
Gonyaulax polyhedra	Cultures supplied by Dr. B. M. Sweeney	Concentrated by low speed centrifugation during night phase
Renilla kollikeri	Santa Barbara Channel	5 mm wide strip from edge of rachis
Vargula hilgendorfii	Vicinity of Chiba, Japan, *via* Dr. F. H. Johnson	Probably air dried. Samples used were brilliantly luminescent when wetted
Vargula tsujii	Collected at underwater light off dock at Catalina Marine Laboratory	Lyophilized after freezing alive and stored over dessicant
Gaussia princeps	Midwater trawls in San Clemente Basin	Fed alive to test fish immediately upon capture
Euphausia pacifica	Same	Same
Gennadus sp.	Same	Same
Ophiopsila californica	Naples Reef, Santa Barbara, California	Arm sections fed fresh to test fish
Stenobrachius californica	Midwater trawls in San Clemente Basin	Frozen alive. Ventral, photophore-containing parts fed after brief thawing

the weight of *V. hilgendorfii* required to induce luminescence capability in non-luminescent fish.

Studies on fluorescence induction

Rate of induction of fluorescence in non-luminescent fish was estimated on one non-luminescent fish (19.5 cm total length) from Bamfield, B. C., fed 361 mg of dried *V. hilgendorfii*. Subsequently three photophores were removed each day and examined with a fluorescence microspectrophotometer (NanoSpec/10S, Nanometrics Inc., Sunnyvale, Cal.) Excitation illumination was from a mercury lamp filtered with UG 1 and BG 12 filters. Fluorescence was recorded at 524 nm.

Behavioral studies

Luminescent responses to mechanical, visual, and electrical stimuli were studied both in luminescent fish from Southern California and in one luminescence-induced fish from north of Cape Flattery, Washington. Single fish were placed in a small

TABLE III

Results of dietary luminescence experiments.

Fish number	Day	Food	Test for luminescence
1	0	*Gonyaulax polyedra*	(−)
	2	*G. polyedra*	
	4	*G. polyedra*	
	9	*G. polyedra*	
	17		(−)
	32	Beef Liver	(−)
	47		(−)
	53	*Vargula higendorfii* (312 mg*)	(−)
	71		(+)
2	0	*Renilla kollikeri*	(−)
	4	*R. kollikeri*	(−)
	15	*R. kollikeri*	(−)
3	0	*Vargula hilgendorfii* (291 mg*)	(−)
	8		(+)
	12	Beef Liver	(+)
	36		(+)
	142		(+)
	0	*Vargula tsujii* (3**)	(−)
	7		(−)
	39	*V. tsujii* (110 mg*)	
	44		(+)
	75		(+)
	107		(+)
	140		(+)
5	0	*Vargula tsujii* (10 mg*)	(−)
	21	*V. tsujii* (63**)	(+)
	70		(+)
6	0	*Gaussia princeps* (317 mg*)	(−)
	25		(−)
7	0	*Gaussia princeps* (28**)	(−)
	1	*G. princeps* (24**)	
	9		(−)
	23		(−)
8	0	*Gaussia princeps* (12**)	(−)
	1	*G. princeps* (30)	
	9		(−)
	23		(−)
	72	*Euphausia pacifica* (28**)	(−)
	73	*E. pacifica* (14**)	
	74	*E. pacifica* (16**)	
	81		(−)
	95		(−)
	103	*Vargula hilgendorfii* (273 mg*)	(−)
9	121		(+)
	0	*Gaussia princeps* (15**)	(−)
	9		(−)
	23		(−)
	72	*Euphausia pacifica* (35**)	
	73	*E. pacifica* (15**)	
	74	*E. pacifica* (16**)	
	81		(−)
	95		(−)
10	0	*Euphausia pacifica*	(−)
	4		(−)
	19	*E. pacifica*	(−)
	38		(−)
	53		(−)
11	0	*Euphausia pacifica*	(−)
	7	*E. pacifica*	(−)
	12	*E. pacifica* + Beef Liver	
	30		(−)
	41	*Vargula hilgendorfii* (343 mg*)	(−)
	51		(+)
12	0	Beef Liver	(−)
	23		(−)
	38	*Stenobrachius californica*	
	50	*Euphausia pacifica*	(−)
	67	*E. pacifica*	(−)
	75	*S. californica*	(−)
	82	*E. pacifica*	(−)
	119		(−)
13	0	*Gennadas sp* (4***, 3 cm animals)	(−)
	9		(−)
14	0	*Gennadas sp* (4, 3 cm animals)	(−)
	9		(−)
	23		(−)
	72	*Euphausia pacifica* (25**)	
	73	*E. pacifica* (13**)	
	74	*E. pacifica* (16**)	
	81		(−)
	95		(−)
15	0	*Ophiopsila californica*	(−)
	20		(−)

* Weight of lyophilized animals. *V. hilgendorfii* was probably sun dried in Japan and not lyophilized.
** Numbers of living animals, other than *V. tsujii*, which had been lyophilized.
*** Number of 3 cm animals.

aquarium entirely within the view of an upward-facing photomultiplier tube (EMI 9781B). Mechanical stimulation was accomplished by a solenoid-driven rod tapping against the aquarium. Photic stimuli were of two sorts. One was a collimated beam from a 1.5 V incandescent lamp directed across the aquarium at right angles to the photomultiplier field of view. The second was one of two models of the photophore pattern of a 14-cm midshipman. One model presented the lateral aspect and the other a ventral view of the fish. These luminous patterns were produced by templates placed over two green-emitting Sylvania "Panelescent Nightlights."

Low voltage A.C. delivered between silver wire grids at each end of the aquarium invariably induced luminescence in luminescence-competent fish and was used as a control test when other methods of stimulation failed.

Results

Evidence for a discontinuous distribution of P. notatus

P. notatus has been described as ranging from Sitka, Alaska, to Baja California (Hart, 1973). The twelve sites listed in Figure 1 and Table I were sampled to establish regions within this species distribution where luminescence occurs. Figure 1 and Table I show presence or absence of fish and the presence or absence of bioluminescence and fluorescence in collected fish. We were unable to find *P. notatus* between Cape Flattery and Cape Medicino. The existence of this hiatus within the distribution of *P. notatus* was confirmed from two other sources. The first, a survey of museum collections, revealed hundreds of specimens of the midshipman from Puget Sound and Southern California waters but only four specimens collected between Cape Mendicino and Cape Flattery. These were two specimens from Winchester Bay, Oregon (Los Angeles County Museum of Natural History and Oregon State University Ichthyological Museum), one from off the mouth of the Columbia River, and one from Coos Bay, Oregon (O.S.U. Ichthyological Museum). The second confirmation came from a series of 660 trawls along the 100-fathom line between Oxnard, California, and Cape Flattery, Washington, whose results were made available by the NOAA Northwest and Alaska Fisheries Center (Rock Fish Study, 1977, unpublished). *P. notatus* was common from Oxnard to approximately Cape Mendicino. No specimens were recorded between Cape Mendicino and Cape Flattery. Thus the prevalence of *P. notatus* in Puget Sound and in Southern California waters is in striking contrast to its paucity along the Oregon coast.

Correlation of fluorescence with bioluminescence in natural populations

As indicated in Table I and Figure 1, fish from the areas examined in this study were tested for the correlation of fluorescence and bioluminescence. All fish able to luminesce were fluorescent. Monterey coastal waters are evidently the northernmost limit of the entirely luminescent population, since 53 fish from the San Francisco region included non-luminescent (8%) and weakly luminescent (38%) as well as normally luminescent fish (54%). All non-luminescent fish from the San Francisco region were non-fluorescent while those with subnormal luminescence had less than maximal fluorescent capacity. North of the Oregon hiatus neither fluorescence nor bioluminescence was seen. South of Monterey, all fish examined were strongly fluorescent and bioluminescent.

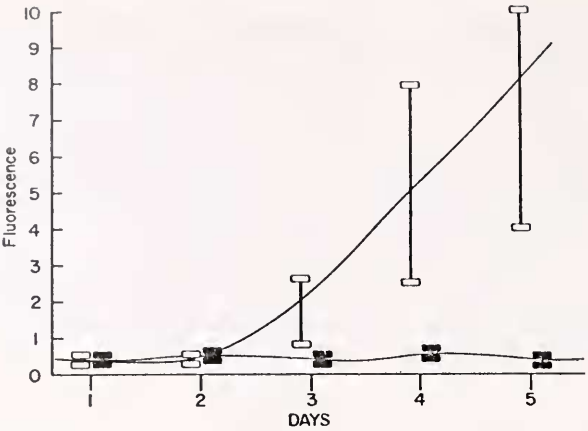

FIGURE 3. The development of fluorescence in a non-luminescent *Porichthys* after feeding with *Vargula*. Each point represents the average of three isolated photophores measured three times. Vertical bars = standard deviation. Upper curve, *Vargula* fed; lower curve, unfed non-luminous fish. Fluorescence scale arbitrary with 10 approximately equal to the typical fluorescence of a southern *Porichthys*.

Fluorescence induction

Onset of photocyte fluorescence was followed in a non-luminescent 19.5-cm fish after feeding with 361 mg of *V. hilgendorfii*. Little or no fluorescence was recordable spectrofluorometrically from isolated photophores before the third day post-feeding. Fluorescence increased markedly from the third to fifth days, when it exceeded the calibrated range of the instrument. During this period the control fish showed no significant amount of photophore fluorescence (Fig. 3).

Dietary induction of luminescence

None of 13 non-luminescent midshipman fish became luminescent when fed upon specimens of eight luminescent organisms sympatric with the Southern California midshipman, as noted in Tables II and III. The four survivors at the end of this experiment were fed approximately 300 mg each of *Vargula hilgendorfii* and all became capable of luminescence upon injection of noradrenaline. In a subsequent experiment, one 19-cm nonluminescent midshipman was fed 110 mg (dry weight; about 700 specimens) of *Vargula tsujii* and developed bright fluorescence and bioluminescence, first noted 4 days after feeding. The ability to luminesce persisted in this fish until its death 120 days later. In this experiment a second nonluminescent, 17.5-cm fish shared the same seawater as the induced fish but was fed beef liver. It developed neither fluorescence nor capacity to luminesce during the induction period for the specimen fed *V. tsujii*. At the conclusion of the experiment this control fish was fed 10 mgs (dry weight; 63 specimens) of *V. tsujii*. Between 15 and 21 days later fluorescence was apparent and the fish luminesced in response to noradrenaline injection. Luminescence capacity persisted until death of the fish 70 days later.

Luminescent capability and behavior

Although Barnes *et al.* (1973) had shown that northern midshipman fish after dietary luminescence induction could be induced to luminesce by electrical stimula-

TABLE IV

Luminescent responses to stimuli

Stimulus type	Number of tests	Percent response	Response characteristics		
			Latency of response (ms)		Duration (seconds)
			Average	Minimum	
A. Southern *Porichthys* (12 fish)					
Light beam	111	26	1300 ± 290*	1,000	5.58 ± 3.50
Model fish	113	30	1030 ± 180	500	4.43 ± 2.36
Mechanical	102	62	660 ± 180	200	7.46 ± 5.40
Electrical	13	84	—	—	11 64 ± 8.41
B. Northern *Porichthys* (1 fish) (First series 82 days after luminescence induction by feeding *Vargula tsujii*)					
Light beam	5	33	1110 ± 160	900	3.41 ± 1.30
Mechanical	12	74	767 ± 98	500	12.00 ± 7.21
(Second series on same fish, 118 days after induction)					
Model fish	19	0	Fish appeared to be blinded, with cornea opaque, perhaps due to fungal attack		
Mechanical	14	71	760 ± 210	300	5.15 ± 2.07

* ± = Standard deviation.

tion, indicating the persistence at least of normal peripheral neural pathways, it is of the greatest interest to determine if the induced northern fish has behavioral control over its artificially endowed capacity to luminesce. To partially answer this question the responses of normally luminescent fish and induced fish to four stimulus types were compared. In order of increasing effectiveness in evoking bioluminescence these were: 1) a midshipman photophore model, 2) a light beam passed through the aquarium, 3) tapping the aquarium side and 4) an A.C. current passed through the aquarium. Table IV sets forth the results of these experiments.

After preliminary tests showed one induced fish to respond to mechanical stimulation, a second fish was carefully examined. Tested with light beam and mechanical stimuli, its responses appeared to fall within the range of normally bioluminescent fish with respect to response latency and rise time (Table IV, Fig. 4). First tested 82 days after an inductive feeding, this fish was left undisturbed and without feeding for an additional 36 days, when its bioluminescent responses to light and mechanical stimuli were found to be still intact. Immediately afterwards it expired following a forced feeding.

DISCUSSION

Discovery that the luminescent ostracod *Vargula tsujii* induces luminescence when fed to nonluminescent *Porichthys* further supports the possibility that a dietary deficiency causes the non-luminescent state of northern *Porichthys*. This

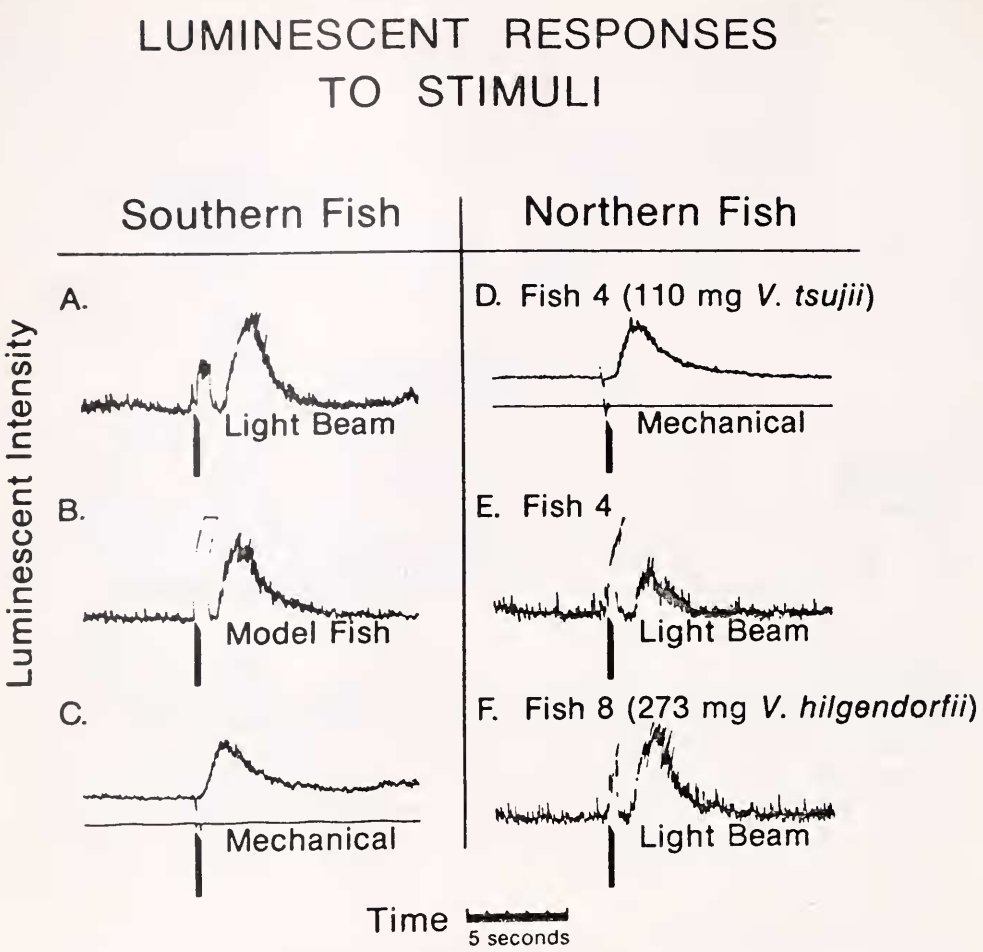

FIGURE 4. Photomultiplier records of behavioral luminescent responses of normally luminescent southern *Porichthys* and *Vargula*-induced northern *Porichthys*.

possibilty is strengthened by the congruence in distributions of the luminescent *Porichthys* population and that of *V. tsujii,* whose northern limit is Monterey Bay (Kornicker and Baker, 1977). This is just south of the zone of incompletely luminescent fish. They also report *V. tsujii* occurring as far south as Bahia de Los Angeles, Baja California, which roughly corresponds to the southern limit of *P. notatus,* according to Miller and Lea (1972). However, no *Porichthys* from waters south of Santa Monica Bay were tested for luminescence in this study.

It is interesting to note that Greene (1899) reported two non-luminous *Porichthys* taken by hook from deep water off Monterey Bay.

Such observations on the overlap in distribution of *V. tsujii* and luminescent populations of *Porichthys,* as well as the inductive capacity of *V. tsujii* when administered to nonluminescent midshipman fish, suggest that all *Porichthys notatus* owe their luminescence to a dietary source of luciferin. However, proof requires evidence of *V. tsujii* or a similarily inductive food source in the *Porichthys* diet and demonstration, by depletion experiments or other means, that this food source is essential to luminescence in fish of the southern population. With respect to the

first point, *V. tsujii* has been taken from the gut of the pomocentrid, *Chromis atrilobata* (Kornicker and Baker, 1977), a fish with feeding habits similar to *P. notatus* (personal communication, A. W. Ebeling, University of California, Santa Barbara) and other ostracods have been found in gut contents of *P. notatus* (Ibara, 1967). It therefore seems possible that *V. tsujii* is part of the diet of the midshipman fish, especially during the juvenile phase when both occupy inshore sandy bottoms.

The fact that the peritoneum was darkly pigmented in all fish examined, including post-larvae as small as 30 mm total length, is also suggestive that the midshipman includes luminescent organisms in its diet. McCallister (1967) proposed that such pigmentation protects against being rendered conspicuous by luminescing food in the digestive tract.

Attempts to deplete luminescence capacity in normally luminescent adult fish have not been successful, even though they have been maintained on beef liver or even starved in laboratory aquaria for more than 5 months (Barnes *et al.,* 1973). In view of the large luciferin stores in body tissues it may be that the southern fish have accumulated more than adequate reserves for life upon reaching maturity. Also they may be able to resynthesize or totally synthesize luciferin. An indication that some dietary luciferin is necessary comes from the fact that it is possible to deplete luciferin reserves sufficiently in southern midshipman larvae evidently to permanently abolish luminescence in specimens kept in a sand and activated-charcoal-filtered seawater supply (Case and Warner, unpublished observations).

Haneda *et al.* (1969) proposed that *V. hilgendorfii,* an ostracod of Japanese waters, is a source of luciferin for *Apogon ellioti* and *Parapriacanthus ransonneti.* They cite the following evidence: 1) all three species have similar luciferins and their luminescent systems cross-react; 2) all three species are sympatric; 3) about 12 *V. hilgendorfii* were found in gut contents of a total of more than 1000 *A. ellioti;* and 4) between gut and light organs in both species of fish there is an opening that is the proposed route of movement of dietary luciferin from gut to luminescent tissues. Luciferin is evidently the only component of the luminescent system that might be required from the diet since the luciferase of *V. hilgendorfii* differs from that of *A. ellioti* (Tsuji and Haneda, 1966).

The arguments in favor of dietary dependence of the midshipman luminescent system are similar to those just listed. Failure to identify *V. tsujii* in the midshipman diet may be only a matter of the small number of specimens examined (recall that only 12 *V. hilgendorfii* were found in 1000 *Apogon*) and the fact that *V. tsujii* was not known at the time of Ibara's (1967) study. Lack of a direct connection between gut and luminescent tissues in the midshipman is not an insurmountable impediment to the hypothesis since the wide distribution of photophores in the midshipman makes such a transport mechanism anatomically improbable. Moreover, there seems to exist a concentrating mechanism appropriate to the midshipman's photophore pattern, namely the microvillous borders of its photocytes. These may be specializations for the uptake of luciferin from the demonstrated large body stores (Anctil, 1979; Case and Strause, 1979; Tsuji *et al.,* 1971).

Our work shows that small amounts of *V. tsujii* induce luminescence capacity which persists for long periods, suggesting that relatively infrequent feeding could maintain sufficient luciferin levels in nature. This finding confirms observations by Barnes *et al.* (1973) on the feeding of *Vargula hilgendorfii* to northern midshipman fish.

The amount of luciferin required to induce luminescence capability in the northern midshipman is vanishingly small. Only 9 μg of purified *Vargula hilgendorfii* luciferin induced prolonged luminescence capability in an adult fish weighing approximately 59 gm (Barnes *et al.,* 1973). In the present study 10 mg of dried *Vargula hilgendorfii* fed to a fish of 74 gm (final frozen weight) was effective. Although the ineffectiveness of oxidized luciferin and several luciferin derivatives in induction of luminescence capacity (Barnes *et al.,* 1973; Tsuji *et al.,* 1975) suggests that the luciferin molecule is not recycled, in the absence of more direct evidence this possibility cannot be excluded since there are other plausible explanations of the present results. For example, enzyme induction might occur, triggered by luciferin. Or the luciferin accumulating mechanism that possibly is associated with the photocyte microvillous cell membranes (Case and Strause, 1979; Anctil, 1979) might be uniquely activated by luciferin and not by the derivatives tested. If the latter is true, a recycling mechanism confined to the photocytes could not be revealed by systemic administration of metabolic intermediates.

It is difficult to explain the origin of this possible dietary dependence of *Porichthys* on *Vargula* for luciferin. Despite the reported dissimilarity of fish and ostracod luciferases, it is worth considering that the fish luminescent system was initially obtained in its entirety from the ostracod. Subsequent, loss of ability to synthesize luciferin would not be as serious a matter as loss of ability to synthesize the enzyme, owing to the undoubtedly greater difficulty of assimilating an intact protein from the digestive tract. Thus the present state with dependence only on exogenous luciferin might arise.

A major argument against this total-initial-assimilation hypothesis is that the fish would have obtained the biosynthetic elements of the luminescent system before developing photophore and control mechanisms. While it might be possible to conjecture some adaptive value for this interim state, it would seem to be the simpler hypothesis to propose that both the ancestral midshipman and *Vargula* independently evolved luminescent systems which underwent sufficient biochemical convergence to allow the *Vargula* luciferin to function in the midshipman system.

Resolution of the origins of this possible dietary dependence, as well as those suspected in other luminescent systems, would be materially advanced by amino-acid sequencing of the relevant luciferases. While certain of these are distinguishable (Tsuji and Haneda, 1966), the techniques used—chromatography, enzyme kinetics, and immunological studies—do not eliminate the possibility that homologous amino-acid sequences exist among these enzymes.

The 1110-km apparent hiatus along the northwest U. S. coast may stem from the low summer temperature of inshore waters in this region, where there is a summer upwelling of cold water (C. Bond, Oregon State University, personal communication). Average summer temperatues at 10 m along the Oregon coast are about 9°C, which is to be compared with 15°C at Vancouver Island and 15°–18°C for outer San Francisco Bay and the more southern waters covered in this study (Barkley, 1968). Migration of *Porichthys* from deeper water to inshore nesting sites (Arora, 1948) coincides with the onset of upwelling so this cold water might interfere with nesting, courtship, or embryonic development. This does not, however, eliminate the possibility of genetic exchange between northern and southern populations; for unless fish return to the coastal zone of origin to breed, they might intermix during their sojourn in deep water. We do not have enough data to decide whether the midshipman occurs at depths greater than 200 m off the hiatus zone. In other localities they are reported down to 600 m, and a

fisherman at Eureka, Calif., told us of trawling midshipman fish near there at 1000 m.

There is no obvious causal relationship between the distributional hiatus and the wholly non-luminescent population to its north because the non-luminescent state clearly is found in the Monterey to San Francisco Bay region. Considering the geological history of the northwestern continental margins, it would seem plausible to suppose that *Porichthys*, a member of a predominantly tropical family, colonized northern waters during the present warm period or during the last interglacial, about 120,000 years ago. During this period $^{18}O/^{16}O$ ratios in deep sea fossils indicate that water temperatures were several degrees higher than now (Shackleton and Opdyke, 1976). Perhaps breeding populations of the midshipman were continuous along the west coast during this or an earlier warm period. Similarly, *Vargula* may have had a distribution matching that of the midshipman. After establishment of the cooling trend in the northeast Pacific, *Vargula* may have retreated to its present distribution, perhaps contemporaneously with establishment of the *Porichthys* coastal hiatus.

Interesting questions are raised by persistence in the northern population of a luminescence system rendered nonfunctional for lack of luciferin but otherwise morphologically, biochemically, physiologically, and behaviorally intact. Assuming that there is no interchange of individuals between northern and southern populations, there should be little advantage in retaining the non-functional luminescence system by the northern fish. Yet, as we find, even appropriate behavioral control persists. Our observations cannot be interpreted as persistence of anything other than a specific bioluminescence behavioral control system. For example, a non-specific, alarm-type adrenergic arousal is unlikely since bioluminescence is difficult to evoke in the midshipman by violent nonspecific stimulation, as first noted by Greene (1899). Since it is doubtful that the resource investment required for development and maintenance of a nonluminescent system would continue for long without some value to the organism, we suggest that this state is either a recent evolutionary development or that the luminescent system has importance beyond the generation of light. For example, the visible photophore pattern may be a necessary intraspecific recognition sign.

Bioluminescence has been nominated to serve at least 20 biological functions (Buck, 1978). *Porichthys* is implicated in at least three of these: mimicry, warning, and courtship. McCallister (1967) notes references to the possibility that the photophore pattern of the midshipman mimics the luminescence of a ctenophore. Tsuji *et al.*, (1971) suggest that the *Porichthys* pattern of luminescence resembles the light of a small swarm of euphausids. This might allow undetected approach to such a swarm, attract the swarm to the fish, or provide protective mimicry against its own predators. Lane (1967) describes the use of luminescence in aggressive behavior of *Porichthys porosissimus*. Crane (1965) observed a courtship display in an aquarium in which grunting and flashing by a male followed its exposure to a female artificially induced to bioluminesce. The similarity of the bioluminescence emission peak and the wavelenth of maximal visual sensitivity in the midshipman further supports the possibility that bioluminescence is of intraspecific significance (Fernandez and Tsuji, 1976). In addition to these possible behavioral uses it must be obvious that the characteristic, predominantly ventral distribution of photophores in the midshipman evokes the possibility of counterillumination (Clarke, 1963).

We are in no position to confirm any of these proposed functions and, further, we note the curious fact that these fish live and reproduce successfully in Puget Sound and even are found in the open waters off outer Vancouver Island with no luminescence capability. This alone places in question any necessary role in courtship. The other proposed roles might still be reasonable since the Puget Sound environment may differ sufficiently from the open marine environment of luminescent-competent midshipman fish to allow speculation that defensive or counterillumination uses of bioluminescence would be unnecessary there and yet vital in the open sea. The outer Vancouver Island fish might seem to defeat even these arguments for a role for bioluminescence. However, it is possible that this population is not truly established there but is maintained by recruitment from inner Puget Sound. Finally, it must be noted that some behavioral modification in the Puget Sound fish, such as a change from nocturnal to diurnal activity, might have diminished the adaptive value of luminescence. Under such conditions the photophore pattern might still be of value, as suggested above. Nevertheless, north of Cape Flattery, *Porichthys* flourishes while lacking the luminescent capacity which presumably adds to the fitness of its southern counterpart.

We are indebted to the following for much valuable advice and assistance: Peter Anderson, Shane Anderson, B. M. Sweeney, R. Satterlie, B. Sumida, and R. Winquist, all of University of California, Santa Barbara; Milton Boyd, Humboldt State University; T. Black, University of British Columbia; G. Calliet, Moss Landing Marine Laboratory; B. B. Collette, National Marine Fisheries Service; Robert Givens, University of Southern California; Donald Gunderson, University of Washington; the late Carl Hubbs, Kenneth H. Nealson, and Frederic I. Tsuji, Scripps Institution of Oceanography; Frank H. Johnson, Princeton University; E. D. Lane, Environment Canada; Robert Lavenberg and C. Swift, Los Angeles County Museum of Natural History; Carl Liem, Museum of Comparative Zoology, Harvard University; John E. McInerney, Bamfield Marine Laboratory; Brad Meyers, Los Angeles; Robert Morris, University of Oregon; A. Paden, British Columbia Provincial Museum; C. Levitt Smith, American Museum of Natural History; Robert Tasto and Paul Riley, California Fish and Game; and Arthur D. Welander, University of Washington.

Our research was supported by Office of Naval Research Contract NOOO14-75-C-024 and University of California Faculty Research Funds.

Summary

Non-luminous northern midshipman fish, members of the species *Porichthys notatus,* are shown to exist as two disjunct populations, one previously known from the Puget Sound area, and the other in waters near San Francisco, where nearly half of the fish collected are non-luminescent or exhibit diminished luminescent capacity. The San Francisco population represents the northernmost occurrence of the southern *Porichthys notatus* population, in which non-luminescent fish are rare or absent. Between the San Francisco and Puget Sound populations a zone between Capes Flattery and Mendicino appears to completely lack midshipman fish in near-shore waters. The distribution of the luminescent ostracod, *Vargula tsujii,* corresponds to that of the luminescent southern fish in that its northernmost extension is in the Monterey-San Francisco region. Northern, non-luminescent

midshipman fish are rendered luminescent by feeding with *Vargula tsujii*. Feeding with seven other luminescent organisms that might contribute to the *Porichthys* diet did not induce luminescence in northern *Porichthys*. The presence of ultraviolet-excited, 524-nm photophore fluorescence was strongly correlated with luminescence capability in both natural populations and experimentally fed fish. Northern midshipman fish in which luminescence capability was induced by feeding *Vargula* were shown to still posses a behavioral luminescence control system indistinguishable from normally luminescent fish. The bearing of these observations on the origin of bioluminescence and its significance in the life of the midshipman fish are discussed.

LITERATURE CITED

Anctil, M., 1977. Development of bioluminescence and photophores in the midshipman fish, *Porichthys notatus*. J. Morphol., **151**: 363–395.

Anctil, M., 1979a. Ultrastructural correlates of luminescence in *Porichthys* photophores. I. Effects of spinal cord stimulation and exogenous noradrenaline. *Rev. Can. Biol.*, **38**: 67–80.

Anctil, M., 1979b. Ultrastructural correlates of luminescence in *Porichthys* photophores. II. Effects of metabolic inhibitors. *Rev. Can. Biol.*, **38**: 81–96.

Anctil, M., and J. F. Case, 1976. Pharmacological study of denervation induced by 6-hydroxydopamine in *Porichthys* photophores. *Cell Tissue Res.*, **166**: 365–388.

Arora, H. F., 1948. Observations on the habits and early life history of the batrachoid fish, *Porichthys notatus* Girard. *Copeia*, **1948**: 89–93.

Baguet, F., 1975. Excitation and control of isolated photophores of luminous fishes. *Prog. Neurobiol. (Oxf.)*, **5**: 97–123.

Baguet, F., and J. F. Case, 1971. Luminescence control in *Porichthys* (Teleostei): Excitation of isolated photophores. *Biol. Bull.*, **140**: 15–27.

Baguet, F., and A. M. Ziets-Nicolas, 1979. Fluorescence and luminescence of isolated photophores of *Porichthys*. *J. Exp. Biol.*, **78**: 47–57.

Barkley, R. A., 1968. *Oceanographic Atlas of the Pacific Ocean*. University of Hawaii Press, Honolulu. 146 pp.

Barnes, A. T., J. F. Case, and F. I. Tsuji, 1973. Induction of bioluminescence in a luciferin deficient form of the marine teleost, *Porichthys*, in response to exogenous luciferin. *Comp. Biochem. Physiol.*, **46**: 709–723.

Buck, J. B., 1978. Functions and evolution of bioluminescence. Pp. 419–473 in P. J. Herring, Ed., *Bioluminescence in Action*. Academic Press, London.

Case, J. F., and L. G. Strause, 1979. Neurally controlled luminescent systems, Pp. 331–365 in P. J. Herring, Ed., *Bioluminescence in Action*. Academic Press, London.

Clarke, W. D., 1963. Function of bioluminescence in mesopelagic organisms. *Nature*, **198**: 1244–1247.

Cormier, M. J., J. M. Crane, Jr., and Y. Nakano, 1967. Evidence for the identity of the luminescent systems of *Porichthys porosissimus* (fish) and *Cypridina hilgendorfii* (crustacean). *Biochem. Biophys. Res. Commun.*, **29**: 747–752.

Crane, J. M., 1965. Bioluminescent courtship display in the teleost *Porichthys notatus*. *Copeia*, **2**: 239–241.

Fernandez, H. R., and F. I. Tsuji, 1976. Photopigments and spectral sensitivity in the bioluminescent fish, *Porichthys notatus*. *Marine Biol.*, **34**: 101–107.

Greene, C. W., 1899. The phosphorescent organs in the toadfish, *Porichthys notatus* Girard. *J. Morphol.*, **15**: 667–696.

Greene, C. W., and H. H. Greene, 1924. Phosphorescence of *Porichthys notatus*, the California singing fish. *Am. J. Physiol.*, **70**: 500–506.

Haneda, Y., F. H. Johnson, and O. Shimomura, 1966. The origin of luciferin in the luminous ducts of *Parapriacanthus ransonneti*, *Pempheris klunzingeri*, and *Apogon ellioti*. Pp. 533–546 in F. H. Johnson and Y. Haneda, Eds., *Bioluminescence in Progress. Princeton University Press*, Princeton, New Jersey.

Haneda, Y., F. I. Tsuji, and N. Sugiyama, 1969. Luminescent systems in apogonid fishes from the Philippines. *Science*, **165**: 188–190.

Hart, J. L., 1973. *Pacific Fishes of Canada*. Bull. 180, Fisheries Res. Board of Canada. 740 pp.

Ibara, R. M., 1967. Biology of the midshipman fish, *Porichthys notatus* Girard. M.A. Thesis, University of California, Santa Barbara, California.

Kornicker, L. S., and J. H. Baker, 1977. *Vargula tsujii*, a new species of luminescent ostracoda from Lower and Southern California (Mycopoda: Cypridinae). *Proc. Biol. Soc. Wash.*, 90: 218–231.

Lane, E. D., 1967. A study of the Atlantic Midshipman, *Porichthys porosissimus*, in the vicinity of Port Aransas, Texas. *Contrib. Mar. Sci.*, 12: 1–53.

McCallister, D. E., 1967. The significance of ventral bioluminescence in fish. *J. Fish. Res. Board Canada*, 24: 537–554.

Miller, D. J., and R. N. Lea, 1972. *Guide to the Coastal Marine Fishes of California. Cal. Fisheries Bull., No. 157*, 249 pp.

Nicol, J. A. C., 1957. Observations on photophores and luminescence in the teleost *Porichthys*. *Q. J. Microsc. Sci.*, 98: 179–188.

Shackleton, N. J., and N. D. Opdyke, 1976. Oxygen isotope and paleomagnetic stratigraphy of Pacific core V28–239 late Pliocene to latest Pleistocene. *Geol. Soc. Am. Mem.*, 145: 449–464.

Strum, J. M., 1968. Fine structure of the dermal luminescent organs, photophores, in the fish, *Porichthys notatus*. PhD. Dissertation, University of Washington, Seattle.

Strum, J. M., 1969a. Fine structure of the dermal luminescent organs, photophores, in the fish, *Porichthys notatus*. *Anat. Rec.*, 164: 433–462.

Strum, J. M., 1969b. Photophores of *Porichthys notatus:* ultrastructure of innervation. *Anat. Rec.*, 164: 463–478.

Tsuji, F. I., A. T. Barnes, and J. F. Case, 1972. Bioluminescence in the marine teleost, *Porichthys notatus*, and its induction in a non-luminous form by *Cyprinda* (ostracod) luciferin. *Nature*, 237: 515–516.

Tsuji, F. I., and Y. Haneda, 1966. Chemistry of the luciferases of *Cypridina hilgendorfii* and *Apogon ellioti*. Pp. 137–149 in F. H. Johnson and Y. Haneda, Eds., *Bioluminescence in Progress*. Princeton University Press, Princeton, New Jersey.

Tsuji, F. I., R. V. Lynch, III, and N. Sugiyama, 1971. Luminescent cross reactions of *Porichthys luciferin* and theories on the origin of luciferin in some shallow-water fishes. *Comp. Biochem. Physiol.*, 40: 163–179.

Tsuji, F. I., B. G. Nafpaktitis, T. Goto, M. J. Cormier, J. S. Wampler, and J. M. Anderson, 1975. Spectral characteristics of the bioluminescence induced in the marine fish, *Porichthys notatus*, by *Cypridina* (Ostracod) luciferin. *Mol. Cell. Biochem.* 9: 3–8.

Wilimovski, N. J., 1954. List of the fishes of Alaska. *Stanford Ichthyol. Bull.*, 4: 279–294.

Young, J. Z., 1933. The preparation of isotonic solutions for use in experiments with fish. *Pubbl. Stn. Zool. Napoli*, 12: 425–431.